Fire Blight Resistance of Budagovsky 9 Apple Rootstock

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ABSTRACT

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Erwinia amylovora, the causal agent of fire blight, can cause a fatal infection of apple rootstocks known as rootstock blight. Budagovsky 9 (B.9) apple rootstock is reported to be highly susceptible when inoculated with *E. amylovora*, although results from multiple trials showed that B.9 is resistant to rootstock blight infection in field plantings. Conflicting results could stem from genetic variation in the B.9 population, appearing as phenotypic differences in rootstock material. However, genetic testing, using 23 microsatellite loci, confirmed the clonal uniformity of B.9 in commerce. Variation in growth habit between B.9 rootstocks originating from two nurseries also has been discounted as a source of disease resistance. Instead, results indicate a possible novel resistance phenotype in B.9 rootstock. B.9 rootstock was susceptible to leaf inoculation by *E. amylovora*, statistically similar to the susceptible rootstock Malling 9 (M.9). Conversely, inoculation assays targeting woody 4- to 5-year-old tissue revealed a high level of resistance in B.9, whereas M.9 remained susceptible. Although the mechanism by which B.9 gains resistance to *E. amylovora* is unknown, it is reminiscent of age-related resistance, due to an observed gain of resistance in woody rootstock tissue over succulent shoot tissue. Durable fire blight resistance correlated with tissue development could be a valuable tool for rootstock breeders.

Additional keywords: dwarfing rootstock

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., is a devastating disease of rosaceous plants. Present in over 40 countries, fire blight is a constant threat to apple (*Malus* × *domestica* Borkh.) production worldwide. Most commonly associated with blossom and shoot blight, *E. amylovora* also can cause an infection of the apple rootstock known as rootstock blight (40). Rootstock blight develops rapidly, resulting in an untreatable and lethal infection (20,21,26). In recent years, rootstock blight has generated considerable financial losses due to lost production and cost of replanting (20). Most years, rootstock blight is sporadic, resulting in isolated tree death; however, under severe fire blight conditions, tree losses of 50% and greater have been reported (7,31).

Increased incidence of rootstock blight can be attributed to the use of susceptible dwarfing rootstocks in high-density orchards. High-density orchards require less land, accelerate cropping, generate higher cumulative yields, and produce a greater percentage of premium fruit, providing an economic advantage in a competitive industry (6,11,32). Malling 9 (M.9) rootstock, the industry standard dwarfing rootstock, is particularly susceptible to rootstock blight. Robinson et al. (31) and Ferree et al. (7) reported significantly higher levels of tree mortality for M.9 rootstock compared with rootstock selections moderately or fully resistant to fire blight. Fire-blight-resistant apple rootstocks are the only known method of preventing rootstock blight. Once bacteria breach the plant surface, no cultural or chemical control can prevent disease development (26).

Dwarfing rootstocks conferring desirable horticultural traits and disease resistance are crucial for the advancement of the apple industry. Budagovsky 9 (B.9) is a lesser known but increasingly popular dwarfing rootstock similar in size and productivity to M.9 (2). Historically, B.9 had been reported as susceptible to fire blight (4,5) when inoculated as a nongrafted rootstock. However, B.9 has exhibited a significant level of rootstock blight resistance when scions grafted to it are naturally infected or artificially inoculated in the field (7,26,31,34,36). A wide range of resistance to fire blight has been observed in *Malus* spp., from moderately susceptible to highly resistant; however, the level of that resistance remains constant throughout the life of the plant (15). B.9 rootstock is unique in its widely divergent resistance ratings. Norelli et al. (26) conducted experiments simultaneously comparing infection of shoot-inoculated, nongrafted rootstocks with rootstock blight development in blossom-inoculated grafted trees. Results indicated that B.9 rootstock was susceptible to shoot inoculation but resistant to rootstock blight development as grafted trees. A contradictory report by Travis et al. (39) reported that grafted B.9 rootstock was susceptible to fire blight; however, susceptibility was based on the progression of scion lesions into rootstock tissue and not as an isolated rootstock canker, which is a more diagnostic symptom of rootstock blight. These conflicting reports of B.9 resistance prevent confident recommendation of B.9 rootstock (15,26). If B.9 were, in fact, susceptible as a nongrafted plant but resistant when acting as a grafted rootstock, it would be the first apple rootstock cultivar to demonstrate conditional fire blight resistance.

B.9 rootstocks are propagated mainly at two nurseries, one in the United States (B.9-OR), and one in the Netherlands (B.9-NE). Both nurseries provide rootstocks for the American market, although rootstock origin often is undisclosed at the time of sale. Phenotypic variation in B.9 plants from the different nurseries has been widely reported in the horticultural community (34). Typically, B.9 rootstocks propagated in the Netherlands have flatter branches and more weeping growth pattern than B.9 rootstocks originating from the United States. Because B.9 is clonally propagated, phenotypic variation may have originated from a mutation early in the commercialization process, either in the United States or Europe. Such phenotypic differences in clonally propagated rootstocks are well known, with more than 20 strains of the common M.9 rootstock (42), but no strain distinction has been made for B.9 from either nursery.

Genetic differences in B.9 rootstock sold in the United States may account for variation in both morphology and fire blight resistance, clarifying contradictory reports of B.9's fire blight resistance. Routine examinations of germplasm collections often reveal duplicate selections and otherwise misidentified apple cultivars (19). Misidentification often occurs as a

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result of uncontrolled open pollination, handling mistakes, and incorrect labeling (29). Coupled with the close relatedness and phenotypic similarity of most rootstock clones, genetic verification of plant material is often necessary. Simple sequence repeats (SSRs) have been used extensively to verify the genetic relationships among *Malus* spp. and hybrids. SSR markers are polymerase chain reaction (PCR) based, polymorphic in nature, and easily reproducible. Distributed across the *Malus* genus, SSRs facilitate the comparison of genetic relatedness among *Malus* spp. (9,12,13). Guilford et al. (10) differentiated 21 closely related apple cultivars using a minimum of three SSR markers. Over 200 SSR sequences are currently available for genetic fingerprinting of apple (9,10,12,13,16,27,37).

Although B.9 has been shown to be susceptible to fire blight as a nongrafted plant and resistant as a grafted tree, it is not known if this resistance is due to observed genetic variation between rootstock sources or to a novel resistance mechanism. The objectives of this study were to clarify conflicting reports dealing with the resistance of B.9 rootstocks to *E. amylovora* and to better understand the nature of fire blight resistance. Verification of B.9's genetic identity and fire blight resistance will support recommendation of B.9 as a resistant apple rootstock to succeed M.9 in high-density systems. Bacterial migration and resistance assays were performed to determine the effect that phenotypic variants of B.9 had on bacterial movement in vivo and subsequent rootstock colonization.

MATERIALS AND METHODS

Molecular marker analyses. B.9 rootstocks, designated B.9-OR and B.9-NE, were obtained from TRECO, Inc. (Woodburn, OR) and Janssen Bros. Nursery (The Netherlands), respectively. Additional apple (*Malus* spp.) tissue—including three cultivated rootstocks: Malling 8 (M.8), the maternal parent of B.9 (2); M.9; and Robusta 5 (*M.* × robusta) and four wild Asian accessions: two *Malus sieversii* (Ledeb.) M. Roem, 'Niedzwetzkyana' (GMAL 3563.g and GMAL 3781.c), *M. pumila* (Miller) 'Niedzwetzkyana' (PI 589225), and one *M. sylvestris* (L.) Miller, with uncharacteristic red pigmentation (PI 392302)—were acquired through the United States Department of Agriculture–Agricultural Research Service Plant Genetic Resources Unit's collection of *Malus* germplasm in Geneva, NY. The wild Asian *Malus* accessions of diverse genetic background were chosen to represent the unknown paternal parent of B.9, 'Red Flag.'

Total genomic DNA was isolated from young leaves via the Wizard Genomic DNA Extraction Kit (Promega Corp., Madison WI). Additional polysaccharide precipitation of B.9 DNA was conducted according to Rhodes (30). In all, 23 SSR markers distributed over 17 linkage groups of the genus *Malus* were evaluated, including 20 SSR loci previously described by Liebhard et al. (16) (CH02a08z, CH02c02b, CH02c09, CH02c11, CH02d08, CH02g09, CH03a04, CH03d08, CH04c06, CH04c07, CH04g07, CH05d08, CH05e03, CH05e06, and CH05f04), Silfverberg-Dilworth et al. (37) (Hi11a03), and Hokanson et al. (13) (GD12, GD96, GD100, and GD162), as well as three previously undisclosed markers (GD6, GD136, and GD158; S. C. Hokanson, *unpublished data*). SSR markers were amplified in 15-µl PCR reaction mixtures containing 20 ng of genomic template DNA, 2 mM $MgCl_2$, 3 µl of 5× Flexi Buffer (Promega Corp.), 0.04 mM dNTPs, 0.25 units of GoTaq DNA Polymerase (Promega Corp.), and 0.32 µM each primer. PCR reactions were performed at 95°C for 5 min followed by 30 cycles at 95°C for 30 s; primer annealing at 52.3, 57.7, 61.1, or 63.6°C (13,16,37) for 45 s; 72°C primer elongation for 45 s; and a 7-min extension at 72°C. Amplified products were analyzed using an ABI Prism 310 Genetic Analyzer, GeneScan program (Applied Biosystems, Inc.). Band size and allele binning were based on internal size standard (ROX; Applied Biosystems, Inc.) using Genotyper (version 3.7; Applied Biosystems, Inc.). Genetic distance estimates were calculated using a Jaccard coefficient as described by Staub et al. (38) and Landry and Lapointe (14). Cluster analysis and phenogram were computed using the numerical taxonomy program NTSYS-pc, ver. 2.01 (33).

Plant material. In 2002, duplicate orchard plots were planted at two locations at the New York State Agricultural Experiment Station, Geneva. One plot contained grafted and nongrafted plants in 11.4-liter containers with field soil, whereas the second plot was planted in the ground at uniform tree spacing (1 by 3 m). In both plantings, trees were headed back to 56 cm at planting and branches were trained below horizontal in the summer of 2002 to promote early flowering. Four scion cultivars, Gala, Jonagold, Gingergold, and Red Yorking, were planted in combination with three rootstocks—B.9-NE, M.9, and Geneva 16 (G.16)—except for Gingergold, which was not available on G.16. The rootstock B.9-OR was only available grafted to Gala. Twelve scion–rootstock combinations were planted in total. Nongrafted rootstocks B.9-NE, B.9-OR, M.9, and G.16 were planted at the same time as grafted trees under identical conditions in both the container and field plots.

Leaf inoculation of nongrafted rootstocks. Nongrafted rootstocks B.9-NE, B.9-OR, M.9, and G.16 were grown simultaneously under field and greenhouse conditions. In 2003, vigorously growing shoots, approximately 2 to 4 weeks of age, were inoculated by transversely bisecting the two youngest leaves with scissors dipped in a suspension of *E. amylovora* strain E4001a (Nal^rRp^r; 1×10^7 CFU/ml) in potassium phosphate buffer (PPB; 0.05 M) (21,23). Strain E4001a (Nal^rRp^r) was chosen based on its virulence and selective antibiotic markers (24,25). Up to five shoots were inoculated per rootstock, with individual rootstocks as the unit of replication. Control plants were mock inoculated with PPB (0.05 M). When lesions ceased progressing, lesion length was recorded as a percentage of the current year's shoot growth, described as disease severity, and used as a measure of susceptibility (23). Disease severity was compared using the Waller-Duncan K ratio t test using K = 100.

Bacterial migration. The influence of rootstock cultivar on bacterial migration was assessed by inoculating the aboveground portion of grafted and nongrafted trees in the experimental field plot followed by sequential sampling of host tissue for the presence of *E. amylovora*. Due to inconsistent flowering among cultivars in 2003, trees were shoot inoculated on 6 June. Five actively growing shoots per tree were inoculated, bisecting the two youngest leaves transversely with scissors dipped in a suspension of *E. amylovora* strain E4001a (Nal^rRp^r) (5×10^8 CFU/ml) in PPB (0.05 M) (21). Lesion length, described as disease severity, was evaluated as function of the current year's shoot growth to verify cultivar susceptibility. In 2004, flowering was consistent across all cultivars. On 14 May, trees at 60% of full bloom were sprayed with *E. amylovora* strain E4001a (Nal^rRp^r) (1×10^8 CFU/ml). Inoculum concentration was reduced to normalize infection levels from 2003 to 2004, based on more favorable conditions for fire blight infection. Infected blossoms were recorded as a percentage of total blossoms. Trees were sampled when scion lesions ceased progressing, approximately 6 to 8 weeks after inoculation. In 2003 and 2004, 8 and 14 trees, respectively, per scion–rootstock combination were sampled; not all combinations were available for all replicates. Trunks were surface sterilized with 0.5% NaOCl and rinsed with distilled water. Tissue samples, containing the outer bark, xylem, and phloem, weighing between 0.3 and 0.5 g were taken at three points along the trunk: 50 cm above the (scion-rootstock) graft union, 5 cm above the graft union, and 5 cm below the graft union, using a cork borer. The cork borer was sterilized between isolations. Tissue was ground in 2 ml of PPB (0.05 M) and 100-µl aliquots were plated on Luria-Bertani media amended with rifampicin (50 µg/ml) and nalidixic acid (50 µg/ml). Plates were incubated for 48 h at 28°C and subsequently washed with 2 ml of sterile water (21). PCR reactions, 50 µl of total volume, were carried

out using 2.5 µM *E. amylovora*-specific primers A and B (1), 12.5 mM $MgCl_2$, 5 µl of PCR Reaction Buffer (Promega Corp.), 0.1 mM dNTPs, 0.625 units of Taq DNA Polymerase (Promega Corp.), and 10 µl of bacterial sample. Effects of scion and rootstock on the probability of recovering *E. amylovora* at each isolation point, measured as incidence of *E. amylovora*, were evaluated using logistic regression.

Wound inoculation of grafted and nongrafted rootstocks. B.9-NE and B.9-OR rootstocks were evaluated for resistance to wound inoculation by *E. amylovora* on 14 July 2005 and 21 June 2006. In 2006, the field planting was substituted with potted trees due to an incomplete number of replicates. Wounds, 14 mm in diameter made with a cordless drill, were positioned 5 to 10 cm below the graft union for grafted trees and 10 cm above the soil line for nongrafted rootstocks. Wounds were inoculated with 100 µl of *E. amylovora* strain E4001a in PPB (0.05 M) at different inoculum concentrations: 10^5,10^7, and 10^9 CFU/ml in 2005 and 10^7 and 10^9 CFU/ml in 2006. Control plants were wounded and treated with PPB (0.05 M). Wound-inoculated trees and nongrafted rootstocks were 4 and 5 years of age in 2005 and 2006, respectively. Trees were assessed visually for the development of rootstock lesions over the course of the experiment. The development of typical rootstock blight lesions, recorded as fire blight incidence, was based on the appearance of diagnostic symptoms, including the production of bacterial ooze or development of a fire blight canker at the wound site. The proportion of trees that died as a result of inoculation was recorded at the end of the experiment. Effects of rootstock, scion cultivar, and inoculum concentration on the probability of developing a rootstock blight lesion, and on tree mortality, were evaluated using logistic regression.

RESULTS

Microsatellite analysis. All 23 SSR markers generated multiple alleles when amplified with genomic DNA from nine *Malus* accessions. The number of alleles ranged from 3 to 8, with a mean value of 5.8 alleles per individual locus. Cluster analysis using genetic distance estimates based on 23 SSR profiles clearly distinguished the nine *Malus* accessions in this study (Fig. 1). Limited sample size and diversity of *Malus* accessions prevented the segregation into well-defined clusters, although two broad groups could be distinguished. The two *M. sieversii* (GMAL 3563.g and GMAL 3781.c) selections and the *M. pumila* accession formed one group. The second group included both B.9 rootstocks and the Malling rootstocks, M.8 and M.9. Two accessions—Robusta 5, an *M. baccata* (L.) Borkh. open-pollinated cultivar (5), and a red-leaved genotype labeled *M. sylvestris*, a "wild" apple species common in Europe—showed very little genetic similarity to any of the other genotypes analyzed. Based on the uncharacteristic red leaf color, it is probable that the alleged *M. sylvestris* was misidentified during collection. Based on our parameters, B.9-OR and B.9-NE were not genetically disparate (GD = 1.0), indicating a clonal relationship. M.8 was closely related to B.9-OR and B.9-NE (GD = 0.5), verifying the reported parental relationship. None of the wild Asian accessions were closely related to B.9 rootstock, providing no indication as to the paternity of this cultivar.

Bacterial migration. In 2003 and 2004, severe fire blight developed on orchard trees resulting from shoot and blossom inoculation. Because scion cultivar did not influence the detection of *E. amylovora* at any of three isolation points in either 2003 or 2004, findings were grouped by rootstock cultivar. Detection of *E. amylovora* at 50 cm above the graft union was not significantly different for either scion or rootstock in 2003 or 2004, suggesting that *E. amylovora* migrated from the inoculation site regardless of rootstock or scion genotype (Tables 1 and 2). Due to uncontrolled deer feeding and spray drift, controls plants were heavily infected and were removed from the experiment. The utilization of antibiotic-resistant strains ensured that any bacteria cultured on antibiotic-amended media resulted from artificial inoculation.

Results from experimental years varied. In 2003, rootstock cultivar did not signifi-

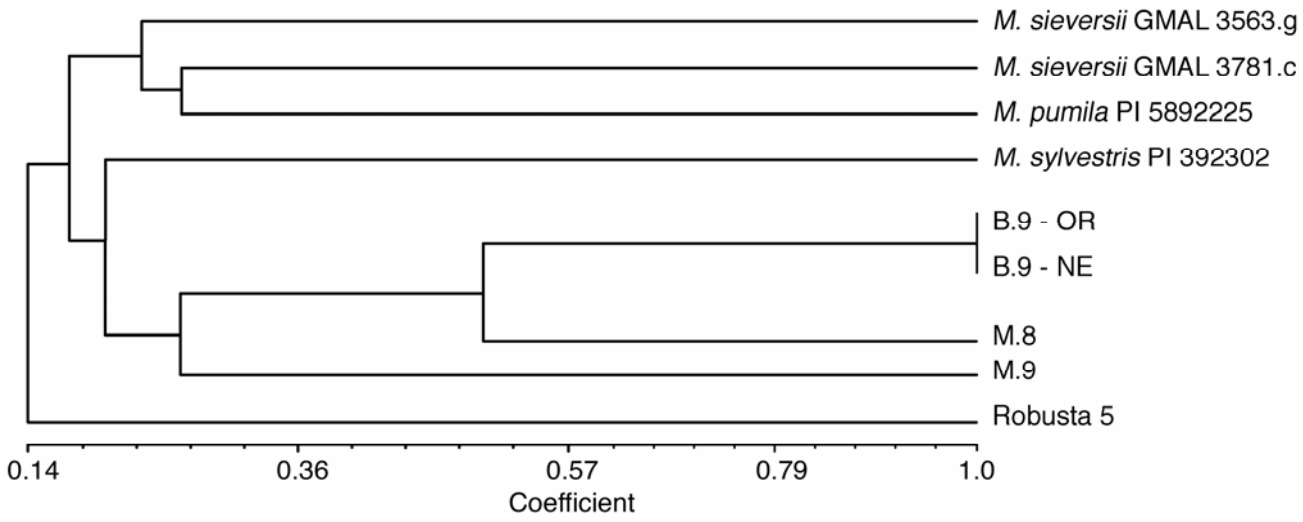

Fig. 1. Genetic distance estimates of nine *Malus* accessions and rootstock cultivars analyzed in this study. Genetic distance coefficient determined using Jaccard analysis.

Table 1. Effect of scion and rootstock on detection of *Erwinia amylovora* at three isolation points in 2003[a]

			50 cm above graft union			5 cm above graft union			5 cm below graft union		
	Df	Res. Df	Deviance	Res. dev.	$P(>\|\chi^2\|)$	Deviance	Res. dev.	$P(>\|\chi^2\|)$	Deviance	Res. dev.	$P(>\|\chi^2\|)$
Null	…	11	…	7.54	…	…	21.89	…	…	7.06	…
Scion	3	8	1.15	6.38	0.76	1.09	20.79	0.78	2.45	4.61	0.48
Rootstock	3	5	1.78	4.61	0.62	7.27	13.53	0.06	0.38	4.23	0.94
Scion × rootstock	5	0	4.61	<0.0001	0.47	13.53	<0.0001	0.02	4.23	<0.0001	0.52

[a] Res. dev. = residual deviance.

cantly affect recovery of *E. amylovora* (Table 1). The only significant effect on *E. amylovora* recovery was the interaction between rootstock and scion cultivar at 5 cm above the graft union (P = 0.02). In 2003, a similar incidence of *E. amylovora* was observed for all rootstock cultivars regardless of fire blight susceptibility or resistance genotype. In 2004, rootstock cultivar did have a significant effect on *E. amylovora* recovery at both 5 cm above (P = 0.01) and 5 cm below the graft union (P = 0.005) (Table 2). No significant interactions between scion and rootstock were identified. In 2004, the susceptible rootstock M.9 had significantly higher incidence of *E. amylovora* recovery than rootstocks B.9-NE, B.9-OR, and the resistant rootstock G.16 (Fig. 2B). Bacteria were detected 5 cm below the graft union in 19% of M.9 rootstocks, a significantly higher incidence of *E. amylovora* than in rootstocks B.9-OR (8%), B.9-NE (0%), and G.16 (0%). *E. amylovora* was not detected in B.9-NE 5 cm above or below the graft union in 2004 (Fig. 2B), nor was it detected 5 cm above the graft union of G.16 rootstocks in 2003 or 2004 (Fig. 2A and B).

Leaf inoculation of nongrafted rootstocks. B.9-NE and B.9-OR displayed characteristic phenotypic differences in both the field and greenhouse experiments. B.9-OR possessed an erect growth type whereas B.9-NE had a spreading growth habit with weeping branch angles. Despite growth differences in B.9 plant material, fire blight sensitivity was similar between B.9-NE and B.9-OR in both field and greenhouse evaluations of nongrafted rootstocks (Fig. 3). Both B.9-NE and B.9-OR were moderately susceptible to fire blight infection and did not differ significantly from each other with regard to disease severity. In the field evaluation, disease ratings were elevated for all rootstocks except M.9, which had significantly less disease than in greenhouse experiments but, at 70% infection, remained highly susceptible to fire blight. B.9-NE had a significantly higher disease severity than B.9-OR in field evaluations; however, neither B.9 rootstock was significantly different from the susceptible control, M.9. No infection was detected in mock-inoculated controls (*data not shown*).

Wound inoculation of grafted and nongrafted rootstocks. Disease symptoms resulting from wound inoculation with *E. amylovora* included the production of bacterial ooze accompanied by dark sunken lesions, which developed over a period of 2 months. Neither inoculum concentration nor scion cultivar significantly affected symptom development in 2005, although scion cultivar was slightly significant in 2006 (Table 3). Based on the high significance of rootstock in both years, treatment and scion cultivar were combined for all rootstocks. Results from the 2 years were consistent, the only irregularity being increased disease incidence in 2006 for all cultivars (Fig. 4B). B.9-NE and B.9-OR did not differ from the resistant rootstock G.16 in either year whereas the susceptible rootstock M.9 had significantly higher disease incidence (Fig 3A and B) and tree mortality (Table 4). Rootstock was the only significant factor affecting tree mortality in either year (P = 0.0001). Rootstocks B.9-NE and B.9-OR and, to a greater extent, G.16 demonstrated the ability to recover from rootstock infection, whereas M.9 had high tree mortality at the end of the experiments in both 2005 and 2006 (Table 4). No significant differences were found between grafted and nongrafted rootstocks with regard to disease development and tree mortality. No symptom development was observed in mock-inoculated controls (*data not shown*).

DISCUSSION

Comparing 23 microsatellite loci, no genetic differences were found between B.9-OR and B.9-NE rootstock material.

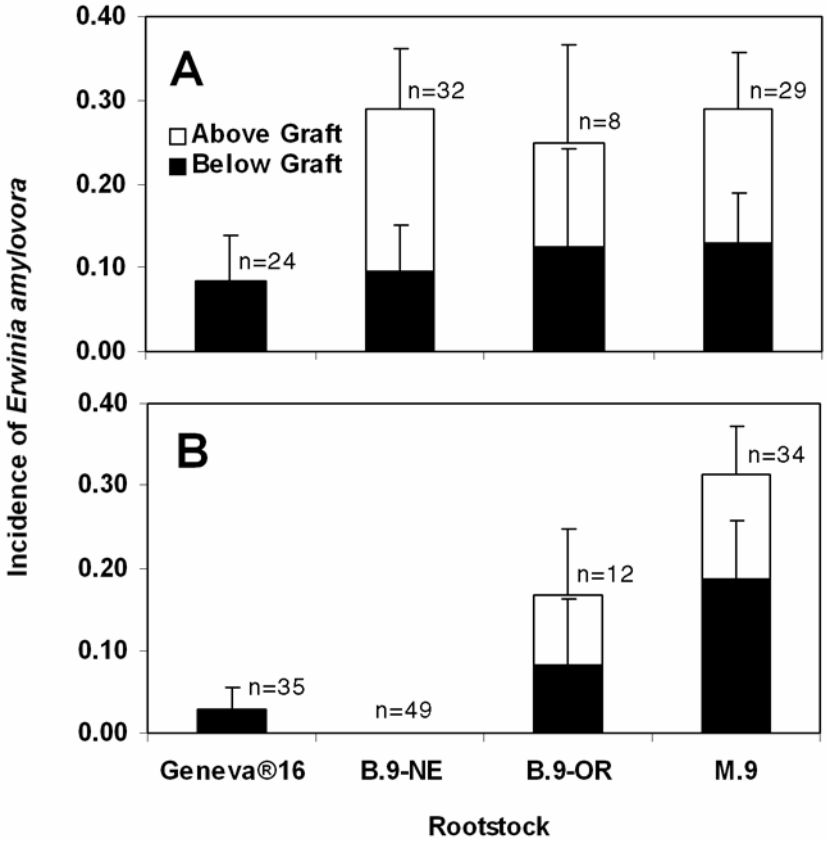

Fig. 2. Proportion of rootstocks that tested positive for *Erwinia amylovora* following shoot or blossom inoculation of the scion in **A,** 2003 and **B,** 2004, respectively. Positive detection based on the presence of 1-kb fragment of ubiquitous *E. amylovora* plasmid pEa29. Trees were assayed 5 cm below the rootstock–scion graft union (solid bars), 5 cm above the graft union (open bars), and 50 cm above the graft union (*data not shown*). Vertical bars represent standard error (+SE). Bacterial presence is indicative of bacterial migration, not symptom development. No rootstock blight symptoms were observed during the 2003 and 2004 seasons.

Table 2. Effect of scion and rootstock on detection of *Erwinia amylovora* at three isolation points in 2004[a]

			50 cm above graft union			5 cm above graft union			5 cm below graft union		
	Df	Res. Df	Deviance	Res. dev.	$P(>\|\chi^2\|)$	Deviance	Res. dev.	$P(>\|\chi^2\|)$	Deviance	Res. dev.	$P(>\|\chi^2\|)$
Null	…	11	…	14.71	…	…	12.38	…	…	16.49	…
Scion	3	8	5.63	9.08	0.13	1.87	10.50	0.60	0.60	15.90	0.90
Rootstock	3	5	3.61	5.47	0.31	10.50	<0.0001	0.01	12.89	3.00	0.005
Scion × rootstock	5	0	5.47	<0.0001	0.36	<0.0001	<0.0001	1.00	3.00	<0.0001	0.70

[a] Res. dev. = residual deviance.

Based on these results, we concluded that B.9 rootstock, propagated independently in Europe and the United States, are equivalent rootstock clones. These results support the sale of B.9 under a single rootstock designation, fostering confidence in the nursery industry. Microsatellite analysis also verified the parental status of M.8 rootstock. Due to a limited sample size, the current study did not provide evidence as to the identity of B.9's parental cultivar, historically referenced as Red Flag (Krasny Shtandard, in Russian) (2). Identification of Red Flag would allow investigation into the genetic basis of B.9's reported resistance to rootstock blight. Further characterization of the apple germplasm collection would impart broader insight into the relationships between popular rootstocks and their "wild" relatives while uncovering novel sources of disease resistance.

Although microsatellite results verified the clonal relationship of B.9 rootstocks sold commercially, they failed to explain the observed phenotypic variation of B.9 rootstocks currently in commerce. Phenotypic variation may be explained through the inadvertent selection of B.9 subclones by different nurseries. A "subclone" is a term used to describe clonal rootstocks selected from within a cultivar for economically relevant attributes. M.9 reportedly has at least 26 subclones that vary in precocity, productivity, and tree vigor (18,41). The genetic bases of differences likely are minor mutations, which previously have proved difficult to identify using microsatellite analysis. Gianfranceschi et al. (9) failed to distinguish 'Red Delicious' and 'Starking,' a somatic mutant with improved color. Monte-Corvo et al. (22) were similarly unsuccessful in the discrimination of five 'Rocha' pear subclones using multiple bioinformatics approaches, despite obvious phenotypic differences. Although subclone selection is important in regards to vigor and stool bed propagation, this study supports previous findings that subclone selection is not linked to significant variation in disease resistance (18,31).

Bacterial migration from localized fire blight lesions into rootstock tissue is a crucial step in the development of rootstock blight (21). Despite differences between 2 years of the current study, bacteria clearly were able to migrate and survive in rootstock tissue for an indeterminate amount of time, regardless of rootstock susceptibility or resistance. The presence of bacteria in resistant rootstocks as well as susceptible cultivars implies that migration into the rootstock is not the critical factor in the development of rootstock blight in B.9 tissue.

Inconsistent results in 2003 and 2004 make it difficult to assess the effect, if any, that phenotypic variants of B.9 exert on bacterial migration. The reason for such seasonal variation is not clear. Weather conditions throughout the experiment were similar with regard to mean monthly temperature and rainfall (Climatological Benchmark Station No. 33031840, Geneva NY). Inoculation method varied between 2003 and 2004, but previous studies have utilized both methods with equivalent results (21,26). The absence of bacteria in B.9-NE rootstocks in 2004 was unexpected given that trees grafted onto B.9-NE suffered severe scion infection and *E. amylovora* was detected 50 cm above the graft union, verifying initial bacterial migration. Failure to detect bacteria in B.9-NE may indicate an underlying effect that divergent growth habits assert on bacterial movement or survival in vivo. There is no conclusive evidence that this variation affects field susceptibility, because no rootstock blight symptoms were observed in B.9 rootstocks from either nursery source during the 2003 and 2004 seasons.

There was general agreement with regard to the level of *E. amylovora* infection between B.9-NE and B.9-OR rootstocks throughout our experiments. Similar levels of fire blight susceptibility or resistance support microsatellite evidence that commercially available B.9 rootstock is clonal in nature. These results also support the conclusion that the two divergent growth forms identified in B.9 do not directly influence resistance to fire blight. Instead, results from two sets of inoculation experiments suggest differentially expressed fire blight resistance when B.9 is leaf inoculated versus wound inoculation of woody rootstock tissue.

Nongrafted, leaf-inoculated B.9-OR and B.9-NE rootstocks displayed high levels of infection in both greenhouse and field experiments, signifying that growth condition does not influence fire blight sensitivity. These results support previous work by Norelli et al. (26), which established B.9 susceptibility when leaf inoculated. Ostensibly, these results validate the classification of B.9 as susceptible to fire blight; however, further investigation has revealed a more complex resistance phenomenon. Although leaf tissue was found to be susceptible to infection by *E. amylovora*, we observed a high level of resistance in B.9 tissue when wound inoculated. When wounds in grafted and nongrafted rootstocks, 4 to 5 years of age, were inoculated directly with *E. amylovora,* B.9 displayed

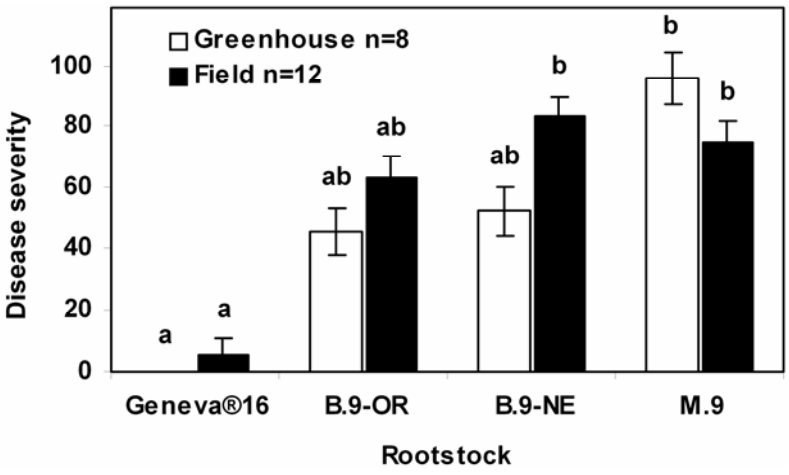

Fig. 3. Disease severity, measured as percent shoot infection, of four nongrafted rootstock cultivars inoculated by bisecting the two youngest leaves. Open bars represent rootstocks inoculated in the greenhouse whereas solid bars represent rootstocks inoculated in field plantings. Mean separation based on Waller-Duncan *k*-ratio. Vertical bars represent standard error (±SE).

Table 3. Effect of scion, inoculum concentration, and rootstock on the development of rootstock blight symptoms following wound inoculation of 4- and 5-year-old grafted and nongrafted rootstocks with *Erwinia amylovora* in 2005 and 2006[a]

	2005				2006			
	Df	Deviance	ML ratio	$P > (\chi^2)$	Df	Deviance	ML ratio	$P > (\chi^2)$
Null	…	31.93	…	…	…	30.04	…	…
Scion	3	35.13	3.20	0.36	3	38.22	8.18	0.042
Rootstock	3	78.92	46.99	<0.0001	3	59.39	29.35	<0.0001
Inoculum concentration	2	36.29	4.36	0.11	1	30.36	0.33	0.57

[a] ML = maximum likelihood.

a level of resistance similar to the resistant rootstock G.16. When similarly inoculated, M.9 rootstock retained its susceptible phenotype, resulting in extensive symptom development and tree mortality. Moreover, symptom development in B.9 tissue, as with G.16, was not indicative of tree mortality, and infections were primarily superficial. Resistance was observed in both grafted and nongrafted B.9 rootstocks, indicating that the grafted phenotype does not directly influence B.9's resistance to rootstock blight. Instead, evidence suggests that the observed resistance is associated with variation in the inoculated tissue, potentially with regard to physiological changes in B.9 related to tissue development or age.

The existence of a novel form of disease resistance in B.9 tissue could explain contradictory experimental findings. Age-related resistance (ARR), or ontogenic resistance, is the phenomenon by which plant tissues gain resistance as either a function of time or relating to a specific phase of tissue development (8,17,28). ARR has been described in many plants, including apple with regard to the apple scab pathogen *Venturia inaequalis*, and is closely correlated with physiological stages of plant development, including the onset of flowering, senescence, or the transition from vegetative to reproductive stage (17,28,35). Based on the evidence presented, it is plausible to suggest that the physiological process of hardening off, or the transition from green tissue to woody tissue, may trigger an innate defense response that could explain B.9's unusual resistance phenotype.

Fire blight resistance in B.9 tissue should not to be confused with the routine loss of fire blight susceptibility that apple tissue exhibits over the course of the growing season (36,43). Fire blight susceptibility is highly correlated with active plant growth; as shoot expansion ceases, apple tissue looses susceptibility. Apple tissue regains susceptibility with the continuation of active growth (43). In contrast, B.9 tissue appears to undergo a permanent reversal of susceptibility.

Regardless of the resistance mechanism, B.9 rootstock tissue displays a high level of resistance to fire blight infection and rarely develops typical rootstock blight symptoms when planted as a grafted or budded tree in commercial or experimental plantings (7,26,31). Based on our results, we can conclude that resistance is due to neither substantial genetic variation in source material nor the inability of *E. amylovora* to migrate into the rootstock. B.9's novel resistance contradicts known fire blight resistance, which has been linked to several quantitative trait loci and is constant throughout the life of the apple tree (3). Not much is known about the parentage of B.9. A thorough investigation of the apple germplasm collection could lead to the discovery of new resistance phenotypes that have been overlooked by the accepted method of fire blight resistance screening. Better understanding of the basis of resistance would be a valuable tool for rootstock breeding programs, providing novel avenues of research in the development of resistant plant material.

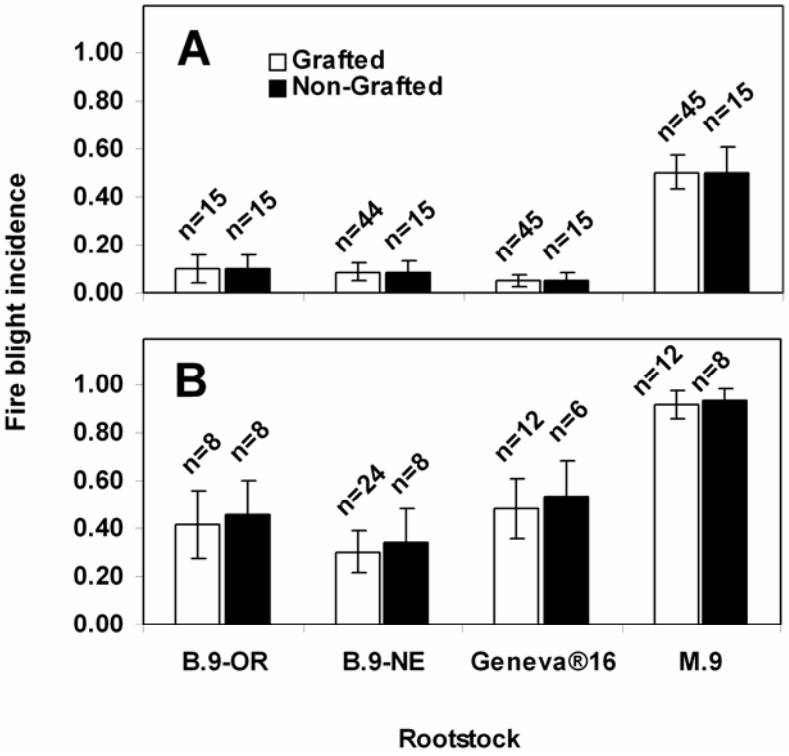

Fig. 4. Proportion of rootstocks that developed rootstock blight symptoms after wound inoculation with *Erwinia amylovora* strain E4001a in **A,** 2005 and **B,** 2006. Open bars represent grafted rootstocks and solid bars represent nongrafted rootstocks. Vertical bars represent standard error (±SE). Symptom development was not a direct measure of tree mortality in either year. Trees planted on Geneva®16, B.9-NE, and B.9-OR did not develop fatal rootstock blight infections.

Table 4. Percent tree mortality resulting from wound inoculation of 4- and 5-year-old grafted and nongrafted rootstocks with *Erwinia amylovora* in 2005 and 2006[a]

	2005		2006	
Rootstocks	**Grafted**	**Nongrafted**	**Grafted**	**Nongrafted**
M.9	15.6 (5.4)	6.7 (6.4)	67.4 (10.4)	86.9 (9.1)
Geneva 16	0	0	3.5 (3.8)	9.7 (9.5)
B.9-NE	0	0	2.4 (2.5)	6.9 (7.2)
B.9-OR	0	0	10.9 (8.0)	26.6 (13.7)

[a] Values in parentheses represent standard errors.

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